200 West Mercer St. • Suite 401 • Seattle, WA 98119
Phone: 206.378.1364 • Fax: 206.217.0089 • www.windwardenv.com

MEMORANDUM

To:	Burt Shephard, EPA Region 10
From:	Shannon Katka, Matt Luxon, John Toll, Windward Environmental LLC
Subject:	Evaluation of TBT Risk to Fish in Portland Harbor
Date:	October 28, 2010
cc:	Eric Blischke, EPA Region 10

This memorandum presents an updated evaluation of tributyltin (TBT) toxicity reference values (TRVs) for use in assessing risk to fish receptors in the Lower Willamette River Study Area. This evaluation builds on the evaluation presented in the draft baseline ecological risk assessment (BERA) (Windward 2009), which was submitted to the US Environmental Protection Agency (EPA) on August 19, 2009. Specifically, additional information for setting the dietary TRV for fish is provided, and as discussed with EPA's Portland Harbor ecological risk assessment lead (Burt Shephard), an updated dietary TRV is recommended by LWG for use in the revised BERA.

1 DIETARY DOSE ASSESSMENT

A TBT dietary dose assessment was presented in the draft BERA (Windward 2009) as directed in EPA's BERA problem formulation (EPA 2008). For this assessment, the daily dose of TBT in prey tissue and sediment (which might be incidentally ingested) from the Study Area were compared with dietary dose no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) TRVs derived from the literature.

The TRV used in the draft BERA (Windward 2009) was based on only one dietary TBT toxicity study (Shimasaki et al. 2003). Although this study was recognized to have significant uncertainty and was regarded as an unreliable basis for estimating a toxicity threshold, it was used in the BERA due to lack of better information. Since the draft BERA was issued, four additional dietary TBT toxicity studies of sufficient quality study design and documentation have been identified (Nakayama et al. 2004; 2005; Nirmala et al. 1999; Shimasaki et al. 2006). These additional studies were not included in

the draft BERA because the literature search conducted for the draft BERA did not return these studies as containing dietary TRVs. Table 1 summarizes the dietary dose calculations, feeding rate assumptions, and shows dietary NOAELs and LOAELs derived from the five studies reviewed.

Dietary dose LOAELs ranged from 0.0020 mg/kg body weight (bw)/day for reduced body weight in juvenile (35 days old at test initiation) Japanese flounder following 65 days of dietary exposure (Shimasaki et al. 2003) to 10 mg/kg bw/day for reduced hatchability and number of viable offspring of Japanese whiting spawned after dietary exposure of both parents to TBT for 3 weeks during the reproductive period (Shimasaki et al. 2006). The NOAELs derived from the studies reviewed ranged from 0.030 to 1.0 mg/kg bw/day.

The LOAEL and NOAEL of 0.0020 and 0.00042 mg/kg bw/day, respectively, calculated from Shimasaki et al. (2003) were used in the draft BERA (Windward 2009) because it was the only study being considered at the time. The NOAEL was extrapolated from the LOAEL using an uncertainty factor of 5. As a basis for the LOAEL, Shimasaki et al. (2003) had significant uncertainty:

- Ingestion rate and body weight were not reported in the study so the calculated doses were based on an assumed ingestion rate of 2% body weight per day.
- Parental fish were experimentally manipulated to produce all female offspring that were subsequently used for the TBT toxicity experiments.
- High mortality was observed in both the control and TBT-exposed groups (survival was 42% in the control group and 57% in the LOAEL group). In standardized fish toxicity tests, control survival less than 90% generally invalidates the test (e.g., ASTM 1996).
- Fish were fed *ad libitum*, but the daily feeding rate was not reported and so was assumed to be 2% of body weight per day.
- The reported LOAEL was 2 to 5 orders of magnitude lower than the LOAELs derived from the other dietary TBT studies identified.

Table 1. Fish dietary TBT toxicity studies

Test Species	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Exposure Conditions	Endpoint: Effect	Source	No-Effect Conc. (mg/kg dw)	Effect Conc. (mg/kg dw)	Body Weight (kg)	Food Ingestion Rate (kg dw/day)	Notes
Japanese flounder	na	0.0020	dietary exposure for 65 days	**growth; sex reversal:** reduced body weight and length (35% reduction in body weight and 12% reduction in length at LOAEL compared with control group); increased sex reversal to male	Shimasaki et al. (2003)	na	0.1	0.001[a]	0.000013[b]	study unreliable because of manipulation of parental fish, high control mortality, and undocumented feeding rate[c]; LOAEL effects measured as statistically different from controls
Japanese medaka	0.030	0.15	maternal exposure via diet for 3 weeks	**reproduction:** reduced swim-up success (10.4% at LOAEL vs. 1.1 and 2.5% at NOAEL and control, respectively) and hatchability (65.4% at LOAEL vs. 97.1 and 93.3% at NOAEL and control, respectively])[d]	Nakayama et al. (2005)	1.0	5.0	0.0003[a]	0.0000090[e]	LOAEL effects measured as statistically different from controls
Japanese medaka	na	1.0	maternal exposure via diet for 3 weeks	**reproduction:** reduced hatching (63% at LOAEL vs. ~85% at control), swim-up (42% at LOAEL vs. ~95% at control), and embryonic success (~80% at LOAEL vs. 95% in control)	Nirmala et al. (1999)	na	100	0.000365[a]	0.000365[f]	effects observed after 2 weeks of exposure; LOAEL effects measured as statistically different from controls
Japanese medaka	na	1.0	maternal exposure via diet for 3 weeks	**reproduction:** reduced fertilization success (46% at LOAEL vs. 96% at control)	Nakayama et al. (2004)	na	33	0.0003[g]	0.0000090[e]	LOAEL effects measured as statistically different from controls

Test Species	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Exposure Conditions	Endpoint: Effect	Source	No-Effect Conc. (mg/kg dw)	Effect Conc. (mg/kg dw)	Body Weight (kg)	Food Ingestion Rate (kg dw/day)	Notes
Japanese whiting	1.0	10	dietary exposure for 30 days	**reproduction:** reduced floating egg rate (83% at LOAEL vs. 94 and 93% at NOAEL and control, respectively), hatchability (82% at LOAEL vs. 91 and 92% at NOAEL and control, respectively, and number of viable larvae (422 at LOAEL vs. 825 and 709 at NOAEL and control, respectively)	Shimasaki et al. (2006)	20	200	0.046[a]	0.0023[h]	LOAEL effects measured as statistically different from controls

[a] Body weight reported in study.

[b] Food ingestion rate estimated as 2% of body weight per day.

[c] All test fish were genetically XX fish (i.e., female) bred from two genetically XX parents though one parent was phenotypically and functionally male. Growth was significantly reduced at 100 days and was recovered after 300 days past hatching (growth not significantly affected at 300 days; however, organisms were only exposed until day 100). Survival was not significantly affected; however, there was high mortality in all groups including control (control survival = 42%; LOAEL survival = 57%).

[d] Exact swim-up success and hatchability percentages were obtained from Oshima et al. (2009), Table 11.2.

[e] Food ingestion rate of 3% of body weight per day was reported in the study.

[f] Food ingestion rate of 1% of body weight per day was reported in the study.

[g] Body weight is based on Nakayama et al. (2005).

[h] Food ingestion rate of 5% of body weight per day was reported in the study.

bw – body weight
dw – dry weight
LOAEL – lowest-observed-adverse-effect level
na – not available
NOAEL – no-observed-adverse-effect level
TBT – tributyltin

The other four studies being evaluated here were better documented and more suitable for use in deriving TRVs for use in the BERA. The relative strengths and weaknesses of these papers are as follows:

- Nakayama et al. (2005) reported a LOAEL and NOAEL of 0.15 and 0.030 mg/kg bw/day, respectively (ingestion rate and body weight were reported in the study so that calculated doses could be verified). Japanese medaka fed 0.15 mg/kg bw/day of TBT for 3 weeks experienced a statistically significant reduction in offspring swim-up success and hatchability compared with those of the control group. The text in Nakayama et al. (2005) reports that swim-up failure was significantly increased in fish fed 0.030 mg/kg bw/day; however, Figure 3b indicates swim-up failure was significantly lower than controls at this concentration. Oshima et al. (2009) presents data from Nakayama et al. (2005) in tabular form that also confirms the significant improvement in swim-up success at the 0.03 mg/kg bw/day dose. Hatchability was not statistically different from controls in fish fed 0.030 mg/kg bw/day. No mortality was reported for adult fish during the 3-week exposure period in either the TBT-exposed groups or the control group, and control offspring hatchability was 90%. Fertility rates of fish were also measured prior to exposure; average fertility rates were >90% in both the control and exposure groups.
- Nirmala et al. (1999) and Nakayama et al. (2004) both reported unbound LOAELs of 1.0 mg/kg bw/day based on statistically significant adverse reproductive effects. Food ingestion rates were reported in both studies. Similar to Nakayama et al. (2005), Nakayama et al. (2004) reported no mortality of adult fish during the 3-week exposure period in either the TBT-exposed groups or the control group, and the average fertility rates of fish measured prior to exposure were >85% in the control and exposure groups. Although observations of adult mortality were not reported by Nirmala et al. (1999),[1] the control group offspring was > 90%.
- Shimasaki et al. (2006) reported a NOAEL and LOAEL of 1.0 and 10 mg/kg bw/day, respectively (ingestion rate and body weight were reported in the study so that calculated doses could be verified). Japanese whiting fed 1.0 mg/kg bw/day of TBT for 30 days experienced a statistically significant reduction in floating egg rate, hatchability, and number of viable larvae compared with those of the control group; no significant effect on offspring swim-up success or hatchability was observed in fish fed 10 mg/kg bw/day. Fish were collected off the coast of Tsuyasaki, Japan, and the exposure levels of fish prior to the experiment were unknown; however, TBT concentrations measured in control group eggs indicated negligible TBT accumulation. Although observations of

[1] Although the study did not report mortality, "no adverse appearance" in adult fish was noted during the 3-week exposure period.

adult mortality were not reported by Shimasaki et al. (2006), the control group offspring hatchability was > 90%.

The LOAELs associated with reproductive effects as reported in these four additional studies have substantially less uncertainty associated with them than does the LOAEL reported by Shimasaki et al. (2003):

- Ingestion rate and body weight were reported in the study so that calculated doses could be verified.
- Reproductive effects observed in all four studies were based on paired genetically male and female adults.
- Nakayama et al. (2004) and Nakayama et al. (2005) reported no mortality in any of the TBT-exposed or control groups, and reproductive endpoints measured in the other two studies (Nirmala et al. 1999; Shimasaki et al. 2006) appear to reflect acceptable testing conditions; whereas high mortality was reported in the control group from Shimasaki et al. (2003).
- The LOAEL reported by Nakayama et al. (2005) is bounded, meaning that a NOAEL is also available from the study. Extrapolating a NOAEL from a LOAEL, as was done to derive the NOAEL from Shimasaki et al.(2003), is uncertain.

Because of the documented problems and uncertainty associated with the toxicity data reported in Shimasaki et al. (2003), the LWG recommends that the next lowest NOAEL and LOAELs, which are from Nakayama et al. (2005) should be used in the revised BERA for the evaluation of dietary risks to fish from TBT. TTCs and TSCs derived using the NOAEL and LOAEL from Nakayama et al. (2005) should be used to screen prey tissue and sediment data and derive hazard quotients (HQs) in the revised BERA.

2 TISSUE-RESIDUE ASSESSMENT

No changes to the tissue residue assessment of TBT for fish are recommended for the revised BERA. In the fish tissue-residue assessment line of evidence (LOE), whole-body concentrations of selected fish receptors were compared with tissue concentration thresholds from the literature. Meador argues that the residue effects methodology is the preferred method for assessing TBT toxicity because the range of body tissue concentrations across a wide variety of organisms is small in comparison to the range of exposure concentrations based on ambient media (Meador 2000, 2006).

A tissue TBT screening value of 49.9 µg/kg wet weight (ww) was used in the draft BERA (Windward 2009) per EPA.[2] This value was derived by multiplying the ambient water quality criteria (AWQC) value of 0.073 µg/L (EPA 2002) by a bioconcentration

[2] The approach for developing aquatic tissue-residue screening-level benchmarks was developed by EPA and its partners (EPA 2005) for data evaluation in the ecological preliminary risk evaluation (Windward 2005). This approach is described in Attachment 5 of the draft BERA(Windward 2009).

factor[3] (BCF) of 693. The LWG agrees that this conservative tissue threshold is acceptable for screening tissue. As presented in the draft BERA, all fish tissue TBT concentrations were below the tissue screening threshold so TBT was not identified as a COPC for this LOE.

3 SURFACE WATER ASSESSMENT

No changes to the surface water assessment of TBT and other butyltins for fish are recommended for the revised BERA. The TBT freshwater aquatic life acute criterion used as a screening value was based on acute toxicity data for 12 genera, including 6 genera of fish. The 5th percentile of genus mean acute values (i.e., the final acute value [FAV]) was 0.92 µg/L. The chronic criterion (0.072 µg/L) used as a screening value in the screening level ecological risk assessment was the FAV divided by the acute to chronic ratio of 12.69 (EPA 2003). The LWG agrees that this chronic water threshold is acceptable for screening surface water TBT concentrations. As presented in the BERA, no TBT surface water samples exceeded the TBT water screening value so TBT was not identified as a COPC for this LOE. Study Area TBT concentrations were approximately an order of magnitude or more below the screening value.

4 CONCLUSIONS

Given the uncertainties associated with the dietary TBT fish TRVs based on Shimasaki et al. (2003) and the additional dietary toxicity data identified subsequent to the draft BERA (as described above), the evaluation of risks to fish from TBT should be presented in the revised BERA using a revised dietary TRV that's based on the next lowest LOAEL, which comes from Nakayama et al. (2005). No changes to the tissue-residue and surface water LOEs will be made in the revised BERA for the evaluation of TBT risks to fish.

5 REFERENCES

ASTM. 1996. Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians. E729-96. American Society for Testing and Materials, Philadelphia, PA.

Devillers J, Bintein S, Domine D. 1996. Comparison of BCF models based on Log P. Chemosphere 33:1047-1065.

Dyer SD, White-Hull CE, Shephard BK. 2000. Assessments of chemical mixtures via toxicity reference values overpredict hazard to Ohio fish communities. Environ Sci Technol 34(12):2518-2524.

EPA. 2002. National recommended water quality criteria: 2002. EPA-822-R-02-047. US Environmental Protection Agency, Washington, DC.

[3] BCFs were based on K_{OW}s for aquatic species developed according the methodology in Dyer et al. (2000) and Devillers (1996), as provided by EPA (2005).

EPA. 2003. Ambient aquatic life water quality criteria for tributyltin (TBT) - final. EPA 822-R-03-031. US Environmental Protection Agency, Washington, DC.

EPA. 2005. EPA comments on technical memorandum: Provisional toxicity reference value selection for the Portland Harbor Preliminary Risk Assessment, March 18, 2005. Oregon Operations Office, US Environmental Protection Agency Region 10, Portland, OR.

EPA. 2008. Problem formulation for the Baseline Ecological Risk Assessment at the Portland Harbor Site. Report and letter dated February 15, 2008 to Lower Willamette Group (from E. Blischke and C. Humphrey to J. McKenna and R. Wyatt). US Environmental Protection Agency Region 10, Oregon Operations Office, Portland, OR.

Meador JP. 2000. Predicting the fate and effects of tributyltin in marine systems. Rev Environ Contam Toxicol 166:1-48.

Meador JP. 2006. Rationale and procedures for using the tissue-residue approach for toxicity assessment and determination of tissue, water, and sediment quality guidelines for aquatic organisms. Human Ecol Risk Assess 12:1018-1073.

Nakayama K, Oshima Y, Yamaguchi T, Tsuruda Y, Kang IJ, Kobayashi M, Imada N, Honjo T. 2004. Fertilization success and sexual behavior in male medaka, *Oryzias latipes*, exposed to tributyltin. Chemosphere 55:1331-1337.

Nakayama K, Oshima Y, Nagafuchi K, Hano T, Shimasaki Y, Honjo T. 2005. Early-life-stage toxicity in offspring from exposed parent medaka, *Oryzias latipes*, to mixtures of tributyltin and polychlorinated biphenyls. Environ Toxicol Chem 24(3):591-596.

Nirmala K, Oshima Y, Lee R, Imada N, Honjo T, Kobayashi K. 1999. Transgenerational toxicity of tributyltin and its combined effects with polychlorinated biphenyls on reproductive processes in Japanese medaka (*Oryzias latipes*). Environ Toxicol Chem 18(4):717-721.

Oshima Y, Nakayama K, Hano T, Gyoon KS, Shimasaki Y, Kang I, Honjo T. 2009. Toxic interactions between tributyltin and polychlorinated biphenyls in aquatic organisms. In: Arai T, Harino H, Ohji M, Langston WJ, eds, Ecotoxicology of antifouling biocides. Springer, Tokyo, Japan, pp 195-206.

Shimasaki Y, Oshima Y, Inoue S, Inoue Y, Kang IJ, Nakayama K, Imoto H, Honjo T. 2006. Effect of tributyltin on reproduction in Japanese whiting, *Sillago japonica*. Mar Environ Res 62:S245-S248.

Shimasaki Y, Kitano T, Oshima Y, Inoue S, Imada N, Honjo T. 2003. Tributyltin causes masculinization in fish. Environ Toxicol Chem 22(1):141-144.

Windward. 2005. Portland Harbor RI/FS. Ecological preliminary risk evaluation. WE-05-0007. Draft. Prepared for Lower Willamette Group. Windward Environmental LLC, Seattle, WA.

Windward. 2009. Portland Harbor RI, Appendix G: Baseline ecological risk assessment. Draft. Prepared for the Lower Willamette Group. Windward Environmental LLC, Seattle, WA.